CELL THERAPY

Can we grow new retina?

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Introduction

Retinal degenerations and dystrophies, the major causes of genetically inherited blindness, are characterised by the death or degeneration of photoreceptors (rods and/or cones).[1] Approaches to treating this disease include: a) replacing the defective gene; b) introducing a drug or agent that either slows down or stops the premature death of photoreceptors; c) introducing electronic chips; or d) replacing the damaged cells by cellular therapy. Gene therapy is aimed at counteracting the defective gene by substituting it with the normal gene in the target tissues. Though successful visual recovery has been reported with gene therapy in dog models,[2-3] it remains a challenge to identify a safe and reliable way of introducing the corrective gene in humans, given that the genes need to act for the lifetime of the patient. Introduction of 'a' factors (such as growth factors) into the eye, directly or through implants, is another novel approach to preventing or slowing premature cell death.[4-5] The challenge lies in delivering the drug to the appropriate site in a safe and sustained manner. Electronic chips, similar to the ones used for audio aids, have shown exciting results in some studies, but the technology is still in its infancy.[6-7]

As knowledge relating to stem cells has increased over the last two decades, attempts have been made to translate this research into clinical practice, particularly for ocular surface reconstruction. Certain ocular surface disorders, like chemical burns, cause damage to the corneal epithelial stem cells. The consequence of this is that the normal corneal epithelium is replaced by conjunctival epithelium, which leads to corneal opacity and vascularisation, with loss of vision. Many centres across the world,[8-10] including our centre,[11-13] have grown sheets of epithelial cells from stem cells, supported on amniotic membrane. These sheets of cells have then been successfully transplanted to cover the entire corneal surface in individuals with ocular surface disorders, leading to less inflammation and scarring. Though the clinical outcome of this new technique is well established, there are still many unanswered questions, particularly in relation to the long-term survival of the transplanted cells.

What are stem cells?

Stem cells are defined as undifferentiated ('primitive') cells that are capable of self-renewal (dividing) and differentiation (changing into cells which have different structural characteristics and function). There are basically two types of stem cells, embryonic and adult stem cells. The embryonic cells are totipotent and pluripotent, with a potential to generate all the types of cells. The adult stem cells are few in number and are located in different parts of the body, like bone marrow, skin, and intestinal mucosa, and serve the purpose of regenerating that particular tissue/cells of the body throughout the life span. It is now known that some of these adult stem cells, in addition to generating cells of their own lineage, can also generate cells of other lineages by a principle called 'transdifferentiation' or 'plasticity'.[14] Bone marrow stromal cells, also called mesenchymal stem cells, are the best example of such cells, as they have the potential to form bone, cartilage, neurons and muscle cells.

What is cell therapy?

Conceptually, cell therapy can be broadly classified into four types:

(a) autologous and homogeneous – i.e. the use of the patient's own adult stem cells to regenerate cells of the same kind, e.g. skin cells, limbal stem cells;

(b) autologous and non homogeneous – i.e. the use of the patient's own cells, but to make cells of a different kind, for example regeneration and remodelling of myocardium after injecting autologous bone marrow-derived stem cells;

(c) allogenic cell therapy – i.e. the use of cells of the same kind, but from a different donor. This requires the use of immunosuppressive drugs to prevent the rejection of cells, e.g. bone marrow transplantation;

(d) embryonic cell therapy – i.e. the use of embryonic cells that have been characterised, isolated and shown to differentuate along the desired cell type only.

This article presents a conceptual approach to cell therapy and a brief review of progress in this field.

Which cells to choose?

Referring to the above classification of cell therapy, in principle, retina could be grown from: a) stem cells within the retina, retinal stem cells (Figure 1); b) stem cells within the eye, but outside the retina, e.g. ciliary body stem cells or retinal pigment epithelial cells; c) from the patient's own tissues, but using non-ocular sources, e.g. bone marrow stromal cells or neural stem cells; d) non-self stem cells, e.g. embryonic stem cells. The use of these cells would depend on their availability/accessibility, the techniques available to grow them, the risks involved in harvesting them without the depletion of the donor cells, success in growing them without altering their nature and transplanting them back into the retina.

The progenitor cells of the retina (neural precursor cells)[15-16] do have the potential to constitutively replace the different cells of retinal-like neurons, photoreceptors, and glial cells. This approach could be useful in developing and studying the pathobiology of the stem cells in health and disease, but the technical difficulty in obtaining these cells and their limited availability could be an issue. The ciliary and iris pigment progenitor cells contain a mitotically quiescent population of neural progenitors that proliferate to make neural stem cells, with a potential for self-renewal.[17-18] Experiments have documented the incorporation of these cells into injured retina, but not normal retina, suggesting that functional integration is possible in damaged tissues. Neural progenitor cells from the brain can restore and survive in the damaged retina,[19] but the major limitation is the source of these cells, which is as rare as, or even rarer than, the retinal cells themselves. It has been shown that bone marrow stromal cells can differentiate into retinal cells in injured rat retina, show functional recovery, and also promote or inhibit retinal angiogenesis.[20-23] Similarly, embryonic stem cells (ESCs) were shown to survive, migrate and integrate into the host retina,[24-25] but they do pose a potential risk of tumour-induction after engraftment.

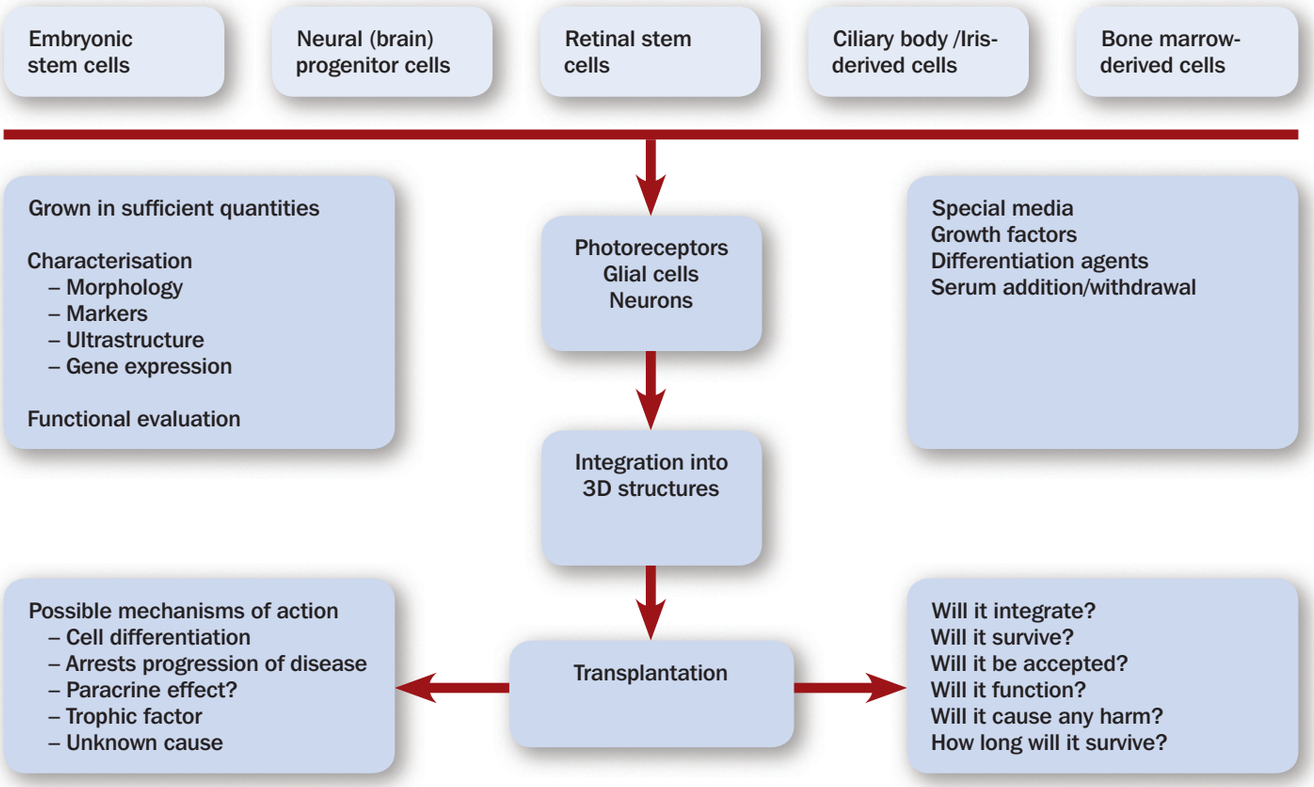
Do we need cells or three-dimensional structures?

The question that now arises is: should we deliver the cells into the target site and hope for the damaged tissue to help in the final integration of the transplanted cells, or should we attempt to organise the cells into tissues before transplantation? A 3D tissue architecture not only provides anatomic integration of cells but also improves the functional outcome.[26] The cells could also be delivered on polymer substrates to the subretinal space, so as to improve their survival, migration and functional restoration.[27]

Will the cells integrate?

Now, assuming the cells and the tissues are ready to generate retina, will they integrate into the adult retina? Unlike the clinical application of cultivated epithelial cells for ocular surface reconstruction, the use of neural cells poses tough challenges. More rigid proof of integration of these cells is warranted. Successful cell therapy should fulfill the following criteria: the desired cells

Figure 1. Thematic approach to growing retinal cells for potential clinical transplantation



should multiply/grow in sufficient quantities, organise into functional units, survive and integrate into the host, and ultimately, function appropriately in a safe manner.

Ethical issues

Clinicians and researchers must constantly deal with the question of adequacy of proof in animal experiments, before moving to human clinical trials. Though a few clinical trials were conducted using foetal cells, there are no published trials using any other cells for clinical transplantation. Reviewing the progress in all fields, it appears that the bone marrow-derived cells have the advantage of being autologous, with proven clinical safety. They could therefore be considered for a pilot study after seeking the approval of regulatory bodies, the Institutional Review Board clearance and patients' informed consent.

Conclusion

In summary, when permanently damaged, the retinal cells which are specialised neurons, cannot be rescued or repaired in the natural process and therefore warrant cell therapy in future.

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Cell therapy glossary

Adult stem cells
Undifferentiated cells found in most adult tissues. Adult stem cells can renew themselves and differentiate to yield all the specialised cell types of the tissue from which they originated. Also referred too as 'somatic stem cells'.

Cell-based therapies
Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or depleted adult cell populations or tissues.

Cellular therapy
A new way to treat disease and injury. It aims to repair damaged and diseased body-parts with healthy new cells provided by stem cell transplants.

Cones
A type of specialised light-sensitive cells (photoreceptors) in the retina that provide sharp central vision and colour vision. See also Rods.

Differentiation
The process whereby an unspecialised early embryonic cell acquires the features of a specialised cell, such as a heart, liver, or muscle cell.

Embryonic stem cells
Primitive (undifferentiated) cells from the embryo that have the potential to become all cell types found in the body (totipotent). Embryonic stem cells (ESCs) are derived from four to five day-old embryos.

Gene therapy
Therapy aimed at counteracting the gene defect by substituting normal gene material at the site of the problem.

Mesenchymal stem cells
Stem cells found primarily in the bone marrow that can transform into bone, cartilage, fat, and connective tissue. These cells are also referred to as bone marrow stromal cells.

Multipotent stem cells
Stem cells that can give rise to several other cell types, but those types are limited in number. An example of multipotent cells is haematopoietic cells – blood stem cells that can develop into several types of blood cells.

Photoreceptors
Cells that are sensitive to light.

Plasticity
The ability of stem cells from one adult tissue to generate the differentiated cell type of another.

Progenitor cells
Cells that can produce only one cell. They can differentiate into a limited number of cell types, but cannot make more stem cells (or renew themselves).

Proliferation
Expansion of a population of cells by the continuous division of single cells.

Regenerative medicine
A treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or depleted adult cell populations or tissues.

Retina
The light-sensitive layer of tissue that lines the back of the eyeball; sends visual messages through the optic nerve to the brain.

Retinal pigment epithelium
The pigment cell layer that nourishes the retinal cells; located just outside the retina and attached to the choroid.

Rods
A type of specialised light-sensitive cells (photoreceptors) in the retina that provide side vision and the ability to see objects in dim light (night vision). Also see Cones.

Stem cells
Unspecialised cells that serve as the source, or 'stem', for specialised cells like heart, brain, or blood cells. They have two important characteristics that distinguish them from other cells in the body. Firstly, they can replenish their numbers for long periods through cell division. Secondly, after receiving certain chemical signals, they can differentiate, or transform into specialised cells with specific functions, such as a heart cell or nerve cell. Found in days-old embryos and a few adult organs.

Subfoveal
Beneath the fovea, the central pit in the macula that produces the sharpest vision.

Undifferentiated cells
Cells that have not changed to become a specialised type of cell.

REPORT

What will be new a

Shaheen Shah reports from the World C

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The World Ophthalmology Congress was held from February 19-24, 2006 in Brazil. I took the opportunity to ask some leading experts how they think the diagnosis and management of posterior segment conditions might be different in the future, specifically in the year 2020. What follows is a summary of their views, which we hope will generate interest and lively discussion amongst our readers and their colleagues.

Diabetic retinopathy

Dr Alexander Brucker, Professor of Ophthalmology at the University of Pennsylvania and Editor of the journal *Retina*, suggests that by the year 2020, decisions about treatment will be based on diagnosis using high definition optical coherence tomography (OCT) visualisation of the retina, in conjunction with fluorescein angiography (FA). Although the interpretation of the clinical findings may be similar, management will be more pharmacologically directed. He anticipates change will also be effected through alteration of the patient's individual risk factor profile. For proliferative disease, the treatment will probably continue to be with panretinal laser photocoagulation, but the addition of new pharmacologic agents (e.g. anti-Vascular Endothelial Growth Factor or anti-VEGF) could reduce the requirement for this destructive treatment.

According to Dr Alistair Laidlaw, Consultant Vitreoretinal Specialist at St Thomas' Hospital, London, UK, the prevention of diabetic retinopathy through effective screening will take priority. He foresees an increased use of non-mydriatic, wide-field, low-light systems, which will make screening comfortable and effective. Management will be through improved medical care of diabetes overall, and use of newer agents (e.g. protein kinase C inhibitors) as well as further developments in non-destructive laser systems.

Retinopathy of prematurity (ROP)

Dr Rajvardhan Azad, Professor of Ophthalmology and Head of Vitreo-Retinal and ROP unit at the Dr R.P. Centre for Ophthalmic Sciences, New Delhi, predicts that by 2020, there will be increased awareness of the condition amongst ophthalmologists and neonatologists through better, easier and more cost-effective imaging of the retina (e.g. RetCam).